



## SEQUENCE LISTING

<110> National University of Singapore  
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Teh, Hsiao Chuin  
Lim, Sze Yun

<120> Oral Vaccine, Method for its Preparation and Use Thereof

<130> 2500-000017

<140> US 10/725,188

<141> 2003-12-01

<160> 9

<170> PatentIn version 3.3

<210> 1

<211> 1810

<212> DNA

<213> Aeromonas hydrophila

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atcctcggcg tgggccatgg cctgttcggc gggttcgctc tgccagcggg tggggccgag 180

gtgatccgac ctcttcttct atttataagg cgagtcgtcg ttattgtgtg ataaatcacc 240

aattcggacg aattttgcca gcggttatcg ctgtaaacgt tttcccatgg cgtgcaaaca 300

atgtgggatt caggtcacaa tttttccgct gtgactatgc ttttcgtaaa aagttccaag 360

tttttttcatt gcggattgga aaacccggtg ctagtctcgg cgccatagtg atgcaaagta	420
catcgctaac acaggaata acaacgactt agtgtttaat tacagtaggc attggaaact	480
atg aaa aag aca att ctg gct att gct atc ccg gct ctg ttt gca tcc Met Lys Lys Thr Ile Leu Ala Ile Ala Ile Pro Ala Leu Phe Ala Ser -20 -15 -10 -5	528
gcc gct aac gct gca gtg gtt tac gac aaa gac ggt acc act ttt gac Ala Ala Asn Ala Ala Val Val Tyr Asp Lys Asp Gly Thr Thr Phe Asp -1 1 5 10	576
gta tac ggc cgt gtt cag gct aac tac tac ggt gac cac aac aaa tct Val Tyr Gly Arg Val Gln Ala Asn Tyr Tyr Gly Asp His Asn Lys Ser 15 20 25	624
gta gct gct acc gat ggt tcc tgg ggc ttc agc gga act ggt acc ccg Val Ala Ala Thr Asp Gly Ser Trp Gly Phe Ser Gly Thr Gly Thr Pro 30 35 40	672
gaa tat act cct ggt acc gct gct cat tac tct gat gtt gat ggt gag Glu Tyr Thr Pro Gly Thr Ala Ala His Tyr Ser Asp Val Asp Gly Glu 45 50 55 60	720
ctg gtt ggt tct tcc cgt ctg ggt tgg tcc ggt aag att gcc ctg aac Leu Val Gly Ser Ser Arg Leu Gly Trp Ser Gly Lys Ile Ala Leu Asn 65 70 75	768
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ggc acc cag tac ggt aag atc atc ttc ggt cag acc gat acc gcg ttc Gly Thr Gln Tyr Gly Lys Ile Ile Phe Gly Gln Thr Asp Thr Ala Phe 110 115 120	912
tat gac gtg ctg gaa ccg acc gat atc ttc aac gag tgg ggc gac gta Tyr Asp Val Leu Glu Pro Thr Asp Ile Phe Asn Glu Trp Gly Asp Val 125 130 135 140	960
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acc tac ggt ggc ttc aaa ggc aaa ctg tcc tat caa acc aac gac gac Thr Tyr Gly Gly Phe Lys Gly Lys Leu Ser Tyr Gln Thr Asn Asp Asp 160 165 170	1056
aag gcc gtc aag gtt act gac gta ggt cag ggc atc aaa gaa aac gca Lys Ala Val Lys Val Thr Asp Val Gly Gln Gly Ile Lys Glu Asn Ala 175 180 185	1104

gtg tac ggc aag gat gtt aag cgt aac tac ggt tat gcc gcg gct gcc Val Tyr Gly Lys Asp Val Lys Arg Asn Tyr Gly Tyr Ala Ala Ala Ala 190 195 200	1152
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gtc tac acc cag gca gat ctg agc tat gac acc acc acc ggt ggt gac Val Tyr Thr Gln Ala Asp Leu Ser Tyr Asp Thr Thr Thr Gly Gly Asp 255 260 265	1344
aag gac aag ggc cgt ggc tac gag ctg gct gct tcc tac aac gtt gat Lys Asp Lys Gly Arg Gly Tyr Glu Leu Ala Ala Ser Tyr Asn Val Asp 270 275 280	1392
gcc tgg act ttc ctg gcc ggc tac aac ttc act gaa ggt aaa gtt gct Ala Trp Thr Phe Leu Ala Gly Tyr Asn Phe Thr Glu Gly Lys Val Ala 285 290 295 300	1440
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 <213> *Aeromonas hydrophila*

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Val Tyr Gly Arg Val Gln Ala Asn Tyr Tyr Gly Asp His Asn Lys Ser  
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Val Ala Ala Thr Asp Gly Ser Trp Gly Phe Ser Gly Thr Gly Thr Pro  
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Glu Tyr Thr Pro Gly Thr Ala Ala His Tyr Ser Asp Val Asp Gly Glu  
 45 50 55 60

Leu Val Gly Ser Ser Arg Leu Gly Trp Ser Gly Lys Ile Ala Leu Asn  
 65 70 75

Asn Thr Trp Ser Gly Ile Ala Lys Thr Glu Trp Gln Val Ser Ala Glu  
 80 85 90

Asn Ser Ala Asn Lys Phe Asp Ser Arg His Ile Tyr Val Gly Phe Asp  
 95 100 105

Gly Thr Gln Tyr Gly Lys Ile Ile Phe Gly Gln Thr Asp Thr Ala Phe  
 110 115 120

Tyr Asp Val Leu Glu Pro Thr Asp Ile Phe Asn Glu Trp Gly Asp Val  
 125 130 135 140

Gly Asn Phe Tyr Asp Gly Arg Gln Glu Gly Gln Ile Ile Tyr Ser Asn  
 145 150 155

Thr Tyr Gly Gly Phe Lys Gly Lys Leu Ser Tyr Gln Thr Asn Asp Asp  
 160 165 170

Lys Ala Val Lys Val Thr Asp Val Gly Gln Gly Ile Lys Glu Asn Ala  
 175 180 185

Val Tyr Gly Lys Asp Val Lys Arg Asn Tyr Gly Tyr Ala Ala Ala Ala  
 190 195 200

Gly Tyr Asp Phe Asp Phe Gly Leu Gly Leu Asn Ala Gly Tyr Ser Tyr  
 205 210 215 220

Ser Asp Leu Glu Asn Thr Ala Thr Asn Asn Thr Gly Asp Lys Lys Glu  
 225 230 235

Trp Ala Leu Gly Ala His Tyr Ala Ile Asn Gly Phe Tyr Phe Ala Gly  
 240 245 250

Val Tyr Thr Gln Ala Asp Leu Ser Tyr Asp Thr Thr Thr Gly Gly Asp  
 255 260 265

Lys Asp Lys Gly Arg Gly Tyr Glu Leu Ala Ala Ser Tyr Asn Val Asp  
 270 275 280

Ala Trp Thr Phe Leu Ala Gly Tyr Asn Phe Thr Glu Gly Lys Val Ala  
 285 290 295 300

Ser Asn Thr Ala Gly Ala Glu Tyr Lys Asp Ile Val Asp Glu Thr Leu  
 305 310 315

Leu Gly Val Gln Tyr Ala Phe Thr Ser Lys Leu Lys Ala Tyr Thr Glu  
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Tyr Lys Ile Gln Gly Ile Asp Lys Met Asp Asp Glu Trp Thr Val Ala  
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Leu Gln Tyr Asn Phe  
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Ala Ala Asn Ala Ala Val Val Tyr Asp Lys Asp Gly Thr Thr Phe Asp	
-1 1 5 10	
gta tac ggc cgt gtt cag gct aac tac tac ggt gac cac aac aaa tct	144
Val Tyr Gly Arg Val Gln Ala Asn Tyr Tyr Gly Asp His Asn Lys Ser	
15 20 25	
gta gct gct acc gat ggt tcc tgg ggc ttc agc gga act ggt acc ccg	192
Val Ala Ala Thr Asp Gly Ser Trp Gly Phe Ser Gly Thr Gly Thr Pro	
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gaa tat act cct ggt acc gct gct cat tac tct gat gtt gat ggt gag	240
Glu Tyr Thr Pro Gly Thr Ala Ala His Tyr Ser Asp Val Asp Gly Glu	
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ctg gtt ggt tct tcc cgt ctg ggt tgg tcc ggt aag att gcc ctg aac	288
Leu Val Gly Ser Ser Arg Leu Gly Trp Ser Gly Lys Ile Ala Leu Asn	
65 70 75	
aac acc tgg tcc ggt atc gcc aag act gag tgg caa gtt tct gct gaa	336
Asn Thr Trp Ser Gly Ile Ala Lys Thr Glu Trp Gln Val Ser Ala Glu	
80 85 90	
aac tcc gcc aac aag ttc gat tcc cgt cac atc tac gtt ggt ttc gac	384
Asn Ser Ala Asn Lys Phe Asp Ser Arg His Ile Tyr Val Gly Phe Asp	
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ggc acc cag tac ggt aag atc atc ttc ggt cag acc gat acc gcg ttc	432
Gly Thr Gln Tyr Gly Lys Ile Ile Phe Gly Gln Thr Asp Thr Ala Phe	
110 115 120	
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125 130 135 140	
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Gly Asn Phe Tyr Asp Gly Arg Gln Glu Gly Gln Ile Ile Tyr Ser Asn	
145 150 155	
acc tac ggt ggc ttc aaa ggc aaa ctg tcc tat caa acc aac gac gac	576
Thr Tyr Gly Gly Phe Lys Gly Lys Leu Ser Tyr Gln Thr Asn Asp Asp	
160 165 170	
aag gcc gtc aag gtt act gac gta ggt cag ggc atc aaa gaa aac gca	624
Lys Ala Val Lys Val Thr Asp Val Gly Gln Gly Ile Lys Glu Asn Ala	
175 180 185	
gtg tac ggc aag gat gtt aag cgt aac tac ggt tat gcc gcg gct gcc	672
Val Tyr Gly Lys Asp Val Lys Arg Asn Tyr Gly Tyr Ala Ala Ala Ala	
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ggt tat gac ttc gac ttc ggt ctg ggt ctg aac gca ggt tac tcc tac	720
Gly Tyr Asp Phe Asp Phe Gly Leu Gly Leu Asn Ala Gly Tyr Ser Tyr	
205 210 215 220	
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Ser Asp Leu Glu Asn Thr Ala Thr Asn Asn Thr Gly Asp Lys Lys Glu	
225 230 235	
tgg gca ctg ggt gca cac tac gcc atc aac ggt ttc tac ttc gcc ggt	816
Trp Ala Leu Gly Ala His Tyr Ala Ile Asn Gly Phe Tyr Phe Ala Gly	
240 245 250	
gtc tac acc cag gca gat ctg agc tat gac acc acc acc ggt ggt gac	864
Val Tyr Thr Gln Ala Asp Leu Ser Tyr Asp Thr Thr Thr Gly Gly Asp	
255 260 265	
aag gac aag ggc cgt ggc tac gag ctg gct gct tcc tac aac gtt gat	912
Lys Asp Lys Gly Arg Gly Tyr Glu Leu Ala Ala Ser Tyr Asn Val Asp	
270 275 280	
gcc tgg act ttc ctg gcc ggc tac aac ttc act gaa ggt aaa gtt gct	960
Ala Trp Thr Phe Leu Ala Gly Tyr Asn Phe Thr Glu Gly Lys Val Ala	
285 290 295 300	
tcc aac acc gct ggt gct gag tac aaa gac atc gtt gac gaa acc ctg	1008
Ser Asn Thr Ala Gly Ala Glu Tyr Lys Asp Ile Val Asp Glu Thr Leu	
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ctg ggc gta cag tac gct ttc act tcc aag ctg aaa gcc tac acc gag	1056
Leu Gly Val Gln Tyr Ala Phe Thr Ser Lys Leu Lys Ala Tyr Thr Glu	
320 325 330	
tac aag atc cag ggt atc gac aag atg gac gac gag tgg acc gtt gcc	1104
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 <212> PRT  
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Val Tyr Gly Arg Val Gln Ala Asn Tyr Tyr Gly Asp His Asn Lys Ser  
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Val Ala Ala Thr Asp Gly Ser Trp Gly Phe Ser Gly Thr Gly Thr Pro  
 30 35 40

Glu Tyr Thr Pro Gly Thr Ala Ala His Tyr Ser Asp Val Asp Gly Glu  
 45 50 55 60

Leu Val Gly Ser Ser Arg Leu Gly Trp Ser Gly Lys Ile Ala Leu Asn  
 65 70 75

Asn Thr Trp Ser Gly Ile Ala Lys Thr Glu Trp Gln Val Ser Ala Glu  
 80 85 90

Asn Ser Ala Asn Lys Phe Asp Ser Arg His Ile Tyr Val Gly Phe Asp  
 95 100 105

Gly Thr Gln Tyr Gly Lys Ile Ile Phe Gly Gln Thr Asp Thr Ala Phe  
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Tyr Asp Val Leu Glu Pro Thr Asp Ile Phe Asn Glu Trp Gly Asp Val  
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Gly Asn Phe Tyr Asp Gly Arg Gln Glu Gly Gln Ile Ile Tyr Ser Asn  
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Thr Tyr Gly Gly Phe Lys Gly Lys Leu Ser Tyr Gln Thr Asn Asp Asp  
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Lys Ala Val Lys Val Thr Asp Val Gly Gln Gly Ile Lys Glu Asn Ala  
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Val Tyr Gly Lys Asp Val Lys Arg Asn Tyr Gly Tyr Ala Ala Ala Ala  
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Gly Tyr Asp Phe Asp Phe Gly Leu Gly Leu Asn Ala Gly Tyr Ser Tyr  
 205 210 215 220

Ser Asp Leu Glu Asn Thr Ala Thr Asn Asn Thr Gly Asp Lys Lys Glu  
 225 230 235

Trp Ala Leu Gly Ala His Tyr Ala Ile Asn Gly Phe Tyr Phe Ala Gly





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 Val Gln Ala Asn Tyr Tyr Gly Asp His Asn Lys Ser Val Ala Ala Thr  
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 Gly Thr Ala Ala His Tyr Ser Asp Val Asp Gly Glu Leu Val Gly Ser  
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 Ser Arg Leu Gly Trp Ser Gly Lys Ile Ala Leu Asn Asn Thr Trp Ser  
 65 70 75 80  
 ggt atc gcc aag act gag tgg caa gtt tct gct gaa aac tcc gcc aac 288  
 Gly Ile Ala Lys Thr Glu Trp Gln Val Ser Ala Glu Asn Ser Ala Asn  
 85 90 95  
 aag ttc gat tcc cgt cac atc tac gtt ggt ttc gac ggc acc cag tac 336  
 Lys Phe Asp Ser Arg His Ile Tyr Val Gly Phe Asp Gly Thr Gln Tyr  
 100 105 110  
 ggt aag atc atc ttc ggt cag acc gat acc gcg ttc tat gac gtg ctg 384  
 Gly Lys Ile Ile Phe Gly Gln Thr Asp Thr Ala Phe Tyr Asp Val Leu  
 115 120 125  
 gaa ccg acc gat atc ttc aac gag tgg ggc gac gta ggt aac ttc tat 432  
 Glu Pro Thr Asp Ile Phe Asn Glu Trp Gly Asp Val Gly Asn Phe Tyr  
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Asp Gly Arg Gln Glu Gly Gln Ile Ile Tyr Ser Asn Thr Tyr Gly Gly	
145 150 155 160	
ttc aaa ggc aaa ctg tcc tat caa acc aac gac gac aag gcc gtc aag	528
Phe Lys Gly Lys Leu Ser Tyr Gln Thr Asn Asp Asp Lys Ala Val Lys	
165 170 175	
gtt act gac gta ggt cag ggc atc aaa gaa aac gca gtg tac ggc aag	576
Val Thr Asp Val Gly Gln Gly Ile Lys Glu Asn Ala Val Tyr Gly Lys	
180 185 190	
gat gtt aag cgt aac tac ggt tat gcc gcg gct gcc ggt tat gac ttc	624
Asp Val Lys Arg Asn Tyr Gly Tyr Ala Ala Ala Ala Gly Tyr Asp Phe	
195 200 205	
gac ttc ggt ctg ggt ctg aac gca ggt tac tcc tac tcc gat ctg gaa	672
Asp Phe Gly Leu Gly Leu Asn Ala Gly Tyr Ser Tyr Ser Asp Leu Glu	
210 215 220	
aat acc gca acc aac aac act ggc gac aag aaa gag tgg gca ctg ggt	720
Asn Thr Ala Thr Asn Asn Thr Gly Asp Lys Lys Glu Trp Ala Leu Gly	
225 230 235 240	
gca cac tac gcc atc aac ggt ttc tac ttc gcc ggt gtc tac acc cag	768
Ala His Tyr Ala Ile Asn Gly Phe Tyr Phe Ala Gly Val Tyr Thr Gln	
245 250 255	
gca gat ctg agc tat gac acc acc acc ggt ggt gac aag gac aag ggc	816
Ala Asp Leu Ser Tyr Asp Thr Thr Thr Gly Gly Asp Lys Asp Lys Gly	
260 265 270	
cgt ggc tac gag ctg gct gct tcc tac aac gtt gat gcc tgg act ttc	864
Arg Gly Tyr Glu Leu Ala Ala Ser Tyr Asn Val Asp Ala Trp Thr Phe	
275 280 285	
ctg gcc ggc tac aac ttc act gaa ggt aaa gtt gct tcc aac acc gct	912
Leu Ala Gly Tyr Asn Phe Thr Glu Gly Lys Val Ala Ser Asn Thr Ala	
290 295 300	
ggg gct gag tac aaa gac atc gtt gac gaa acc ctg ctg ggc gta cag	960
Gly Ala Glu Tyr Lys Asp Ile Val Asp Glu Thr Leu Leu Gly Val Gln	
305 310 315 320	
tac gct ttc act tcc aag ctg aaa gcc tac acc gag tac aag atc cag	1008
Tyr Ala Phe Thr Ser Lys Leu Lys Ala Tyr Thr Glu Tyr Lys Ile Gln	
325 330 335	
ggg atc gac aag atg gac gac gag tgg acc gtt gcc ctg caa tac aac	1056
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Phe	

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<211> 353  
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 <213> Aeromonas hydrophila

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Asp Gly Ser Trp Gly Phe Ser Gly Thr Gly Thr Pro Glu Tyr Thr Pro  
 35 40 45

Gly Thr Ala Ala His Tyr Ser Asp Val Asp Gly Glu Leu Val Gly Ser  
 50 55 60

Ser Arg Leu Gly Trp Ser Gly Lys Ile Ala Leu Asn Asn Thr Trp Ser  
 65 70 75 80

Gly Ile Ala Lys Thr Glu Trp Gln Val Ser Ala Glu Asn Ser Ala Asn  
 85 90 95

Lys Phe Asp Ser Arg His Ile Tyr Val Gly Phe Asp Gly Thr Gln Tyr  
 100 105 110

Gly Lys Ile Ile Phe Gly Gln Thr Asp Thr Ala Phe Tyr Asp Val Leu  
 115 120 125

Glu Pro Thr Asp Ile Phe Asn Glu Trp Gly Asp Val Gly Asn Phe Tyr  
 130 135 140

Asp Gly Arg Gln Glu Gly Gln Ile Ile Tyr Ser Asn Thr Tyr Gly Gly  
 145 150 155 160

Phe Lys Gly Lys Leu Ser Tyr Gln Thr Asn Asp Asp Lys Ala Val Lys  
 165 170 175

Val Thr Asp Val Gly Gln Gly Ile Lys Glu Asn Ala Val Tyr Gly Lys  
 180 185 190

Asp Val Lys Arg Asn Tyr Gly Tyr Ala Ala Ala Ala Gly Tyr Asp Phe  
 195 200 205

Asp Phe Gly Leu Gly Leu Asn Ala Gly Tyr Ser Tyr Ser Asp Leu Glu  
 210 215 220

Asn Thr Ala Thr Asn Asn Thr Gly Asp Lys Lys Glu Trp Ala Leu Gly  
 225 230 235 240

Ala His Tyr Ala Ile Asn Gly Phe Tyr Phe Ala Gly Val Tyr Thr Gln  
 245 250 255

Ala Asp Leu Ser Tyr Asp Thr Thr Thr Gly Gly Asp Lys Asp Lys Gly  
 260 265 270

Arg Gly Tyr Glu Leu Ala Ala Ser Tyr Asn Val Asp Ala Trp Thr Phe  
 275 280 285

Leu Ala Gly Tyr Asn Phe Thr Glu Gly Lys Val Ala Ser Asn Thr Ala  
 290 295 300

Gly Ala Glu Tyr Lys Asp Ile Val Asp Glu Thr Leu Leu Gly Val Gln  
 305 310 315 320

Tyr Ala Phe Thr Ser Lys Leu Lys Ala Tyr Thr Glu Tyr Lys Ile Gln  
 325 330 335

Gly Ile Asp Lys Met Asp Asp Glu Trp Thr Val Ala Leu Gln Tyr Asn  
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Phe

<210> 9  
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 <212> PRT  
 <213> Aeromonas hydrophila strain PPD

<300>  
 <301> SY Lee, Z Yin, R Ge, YM Sin  
 <302> Isolation and characterization of fish Aeromonas hydrophila  
 adhesins important for in vitro epithelial cell invasion  
 <303> Journal of Fish Diseases  
 <304> 20  
 <305> 3  
 <306> 169-175  
 <307> 1997-05  
 <313> (1) .. (20)

<400> 9

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			20